J Vet Sci. 2021 May;22(3):e32
https://doi.org/10.4142/jvs.2021.22.e32
pISSN 1229-845X·eISSN 1976-555X

Journal of Veterinary Science

Rapid Communication
Virology

Check for updates

Full-length ORF2 sequence-based genetic and phylogenetic characterization of Korean feline caliciviruses

Sung Jae Kim [1,†], **Cheongung Kim** [1,†], **Hee Chun Chung** [1], **Yong Ho Park** [1], **Kun Taek Park** [2,*]

[1]Department of Veterinary Microbiology, College of Veterinary Medicine, Seoul National University, Seoul 08826, Korea
[2]Department of Biotechnology, Inje University, Gimhae 50834, Korea

OPEN ACCESS

Received: Sep 6, 2020
Revised: Feb 8, 2021
Accepted: Feb 21, 2021

***Corresponding author:**
Kun Taek Park
Department of Biotechnology, Inje University, 197 Inje-ro, Gimhae 50834, Korea.
E-mail: ktpark@inje.ac.kr

†Sung Jae Kim and Cheongung Kim have contributed equally to this study.

ORCID iDs
Sung Jae Kim
https://orcid.org/0000-0002-8813-012X
Cheongung Kim
https://orcid.org/0000-0003-1205-0876
Hee Chun Chung
https://orcid.org/0000-0003-4666-5393
Yong Ho Park
https://orcid.org/0000-0002-4168-6884
Kun Taek Park
https://orcid.org/0000-0001-6177-0373

Funding
This work was supported by the 2018 Inje University Research Grant.

ABSTRACT

Feline calicivirus (FCV) is a highly infectious pathogen in cats and widely distributed worldwide with high genetic variation. Full-length open reading frame 2 of 5 from recently isolated Korean FCV isolates were sequenced and compared with those of global isolates. The results of phylogenetic analysis supported dividing global FCV isolates into two genogroups (type I and II) and demonstrated the presence of genogroup II in Korea, indicating their geographic spread in East Asia. High sequence variations in region E of the FCV isolates emphasizes that a novel vaccine needs to be developed to induce protective immunity against various FCV strains.

Keywords: Feline calicivirus; phylogenetic analysis; genogroup; open reading frame 2

INTRODUCTION

Feline calicivirus (FCV) is a highly infectious pathogen that generally causes acute, mild-to-moderate stomatitis and upper respiratory tract disease (URTD) in cats worldwide. The FCV genome is a 7.7 kb, positive-sense, single-stranded RNA molecule with three open reading frames (ORFs). ORF1 encodes a polyprotein that is proteolytically cleaved by the viral protease to release the nonstructural proteins, ORF2 encodes the major capsid protein VP1, and ORF3 encodes the minor capsid protein VP2 [1,2]. Parts of ORF2 sequences have been used to elucidate the phylogenetic relationships among FCV isolates [3,4]. Most phylogenetic analyses of nucleotide (nt) sequences from the ORF2 gene result in a “star-like” phylogeny with little statistical support for sub-species clusters. As a result, FCV has been considered to represent multiple strains belonging to a single diverse genotype [5,6]. In contrast, two recent independent studies suggested the presence of two distinct FCV genogroups, putting into question the classification of this virus [3,7]. Considering this question, the aim of this study was to determine the molecular characteristics and phylogenetic relationships of Korean FCV isolates with a comparison to global isolates and to validate the adequacy of phylogenetic classification based on full-length ORF2 sequence homologies.

Conflict of Interest

The authors declare no conflicts of interest.

Author Contributions

Conceptualization: Kim JS, Park YH; Data curation: Kim JS; Formal analysis: Kim JS, Kim C; Funding acquisition: Park KT; Investigation: Kim JS, Kim C; Methodology: Kim JS, Chung HC; Project administration: Park KT; Resources: Kim JS, Kim C; Software: Kim JS, Kim C; Supervision: Park KT; Validation: Kim JS, Kim C; Visualization: Kim JS, Kim C; Writing - original draft: Kim JS, Kim C, Park KT; Writing - review & editing: Chung HC, Park YH, Park KT.

MATERIALS AND METHODS

Viral RNA extraction and complementary DNA (cDNA) synthesis

Five Korean FCV isolates were recently recovered from two hospital-admitted cats with URTD and three healthy stray cats (**Supplementary Table 1**) [8]. Among them, two isolates were collected from hospital-admitted cats with clinical signs, including one (5 yr, strain 121) with mild nasal discharge and the other (3 yr, strain SU) with severe URTD (nasal bleeding, oral ulcer, and conjunctivitis). These adult cats were given a tetravalent vaccine (FHV-1, FCV, FPV and *Chlamydia psittaci*) containing FCV strain F-9 three times at 3-week intervals when they were 6 weeks of age. After these vaccinations, they had not been vaccinated. Three isolates were from healthy adult stray cats (age unknown) and they had never been vaccinated. Viral RNA was extracted from the cell culture supernatants of viral cultures using the viral DNA/RNA extraction kit (Intronbiotech, Korea). cDNA was synthesized using RNA to cDNA EcoDry Premix (Clontech, Japan, Cat no. 639546), which was used for sequencing the full ORF2 region.

Sequencing of full-length ORF2

Full-length ORF2 sequences of the five Korean isolates were obtained with three sets of primers, from a combination of five primers (ORF1-F/ORF2-R, ORF1-F/Cap1R, and Cap1F/ORF3-R; **Supplementary Fig. 1** and **Supplementary Table 2**). Primers Cap1F and Cap1R were previously described [5]. The other three primers were newly designed for this study based on a comparison of the reference sequences retrieved from GenBank (**Supplementary Table 2**). Three overlapping DNA fragments were amplified by polymerase chain reaction (PCR), purified, and sequenced. The full-length ORF2 sequences were then obtained from the results of sequencing.

Phylogenetic analysis

Available sequence data of worldwide FCV isolates were retrieved from National Center for Biotechnology Information GenBank. In total, 43 full-length ORF2 sequences were obtained for phylogenetic analysis (dataset 1). Two additional datasets for partial ORF2 sequences were established according to previously reported strategies [3,7], demonstrating the existence of two genotypes among FCV isolates. Dataset 2 comprised 53 partial ORF2 sequences corresponding to amino acid (aa) residues 288–590 [3] and dataset 3 comprised 59 partial ORF2 sequences corresponding to aa 370–583 [7] (aa position numbers are based on positions in the FCV F9 strain).

Datasets 1, 2, and 3 were first tested by likelihood mapping analysis with 1,000 quartets using IQ-TREE version 1.6.11 to assess the phylogenetic information of the input alignments [9]. Maximum-likelihood (ML) trees of each dataset were constructed after automatic selection with IQ-TREE version 1.6.11. We applied 1000 ultrafast bootstrap (UTBoot) repetitions and the SH-aLRT test for each analysis. SH-aLRT and UTBoot supports were assigned to each branch of the ML trees and the tree was considered reliable when SH-aLRT ≥ 80% and UTBoot ≥ 95% [10].

Pairwise genetic distance (p-distance) analysis

P-distances within 43 full-length ORF2 nt sequences were calculated by MEGA V.7 software [11]. The results are displayed as a frequency distribution histogram of p-distance to examine the possibility of different genogroups among the tested FCV isolates [12].

RESULTS

The full-length ORF2 sequences of all five Korean isolates were successfully sequenced and deposited in the GenBank database (see **Supplementary Table 1** for accession numbers). Likelihood mapping analysis of the three datasets are presented as partitioned triangular graphs (**Fig. 1**). The results revealed that the dataset of full-length ORF2 aa sequences (dataset 1) generated the highest percentage of the sum of the three corner regions and the lowest percentage of the central region among the datasets.

The ML phylogenetic tree based on full-length ORF2 sequences of 43 FCV isolates, including the five new Korean isolates, clearly showed two distinct clusters with high branch support values (SH-aLRT of 100 and UTBoot of 100). The five Korean isolates were also separated into two genogroups (**Fig. 2**). The frequency distribution of the 43 full-length ORF2 sequences using intra- and inter-genotype p-distances generated two well-bounded areas each within the range of 0.008–0.270. Intra-genotype p-distances were ≤ 0.227 and inter-genotype p-distances were ≥ 0.255 (**Supplementary Fig. 2**), which supports the presence of two genotypes among global FCV isolates [12]. The ML phylogenetic trees based on datasets 2 and 3 were also considered reliable with high branch support values (**Supplementary Figs. 3** and **4**). However, the phylogenetic analysis of dataset 3 based on nt sequences misclassified some genotype II strains as genotype I (**Supplementary Fig. 4**).

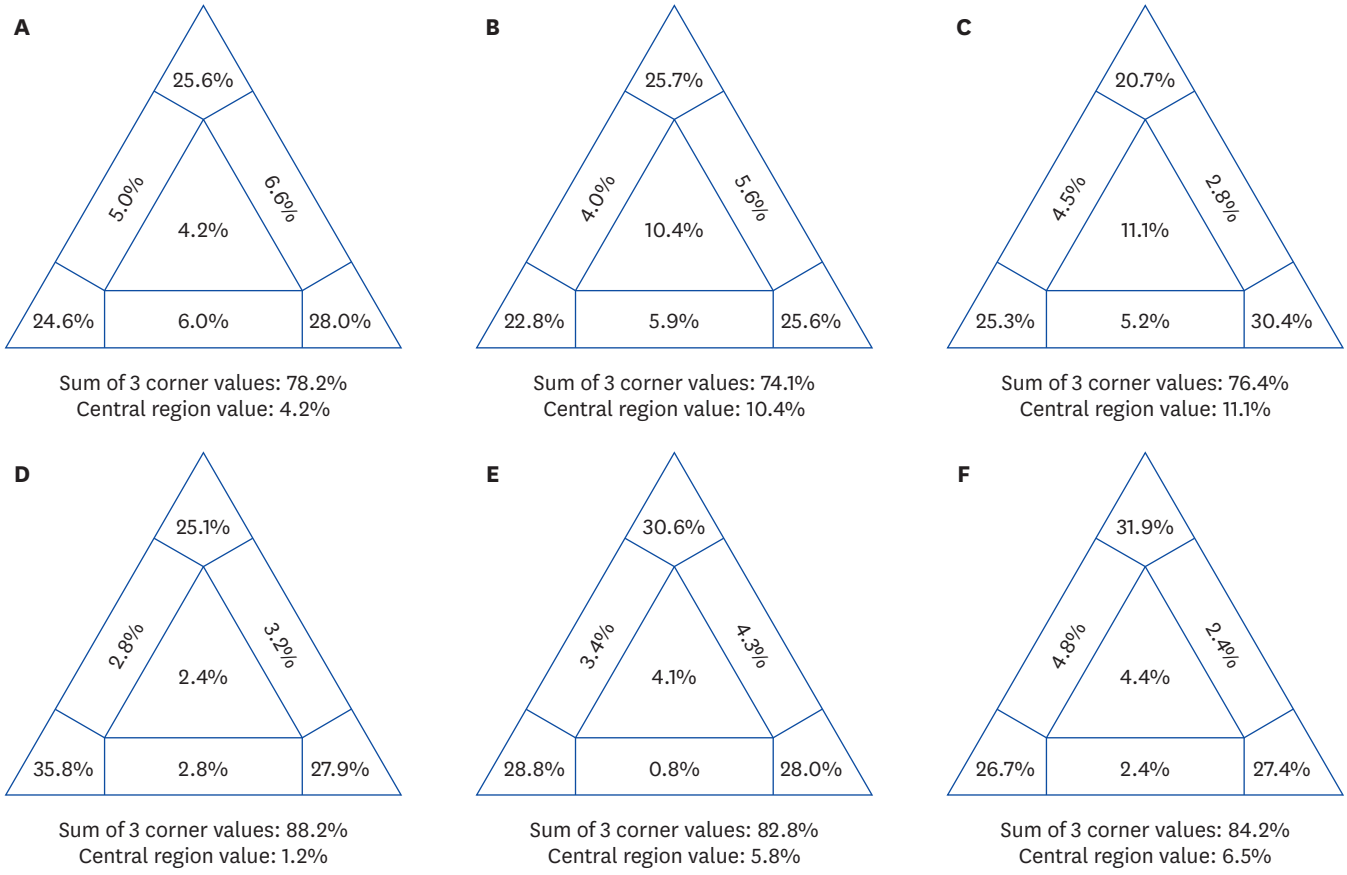

Fig. 1. Likelihood mapping analysis based on full-length ORF2 sequences (dataset 1), partial ORF2 sequences corresponding to amino acid (aa) 288–590 (dataset 2), and partial ORF2 sequences corresponding to aa 370–583 (dataset 3). A, B, and C show the results based on nucleotide sequences of datasets 1, 2, and 3, respectively. D, E, and F show the results based on amino acid sequences of datasets 1, 2, and 3, respectively.
ORF, open reading frame.

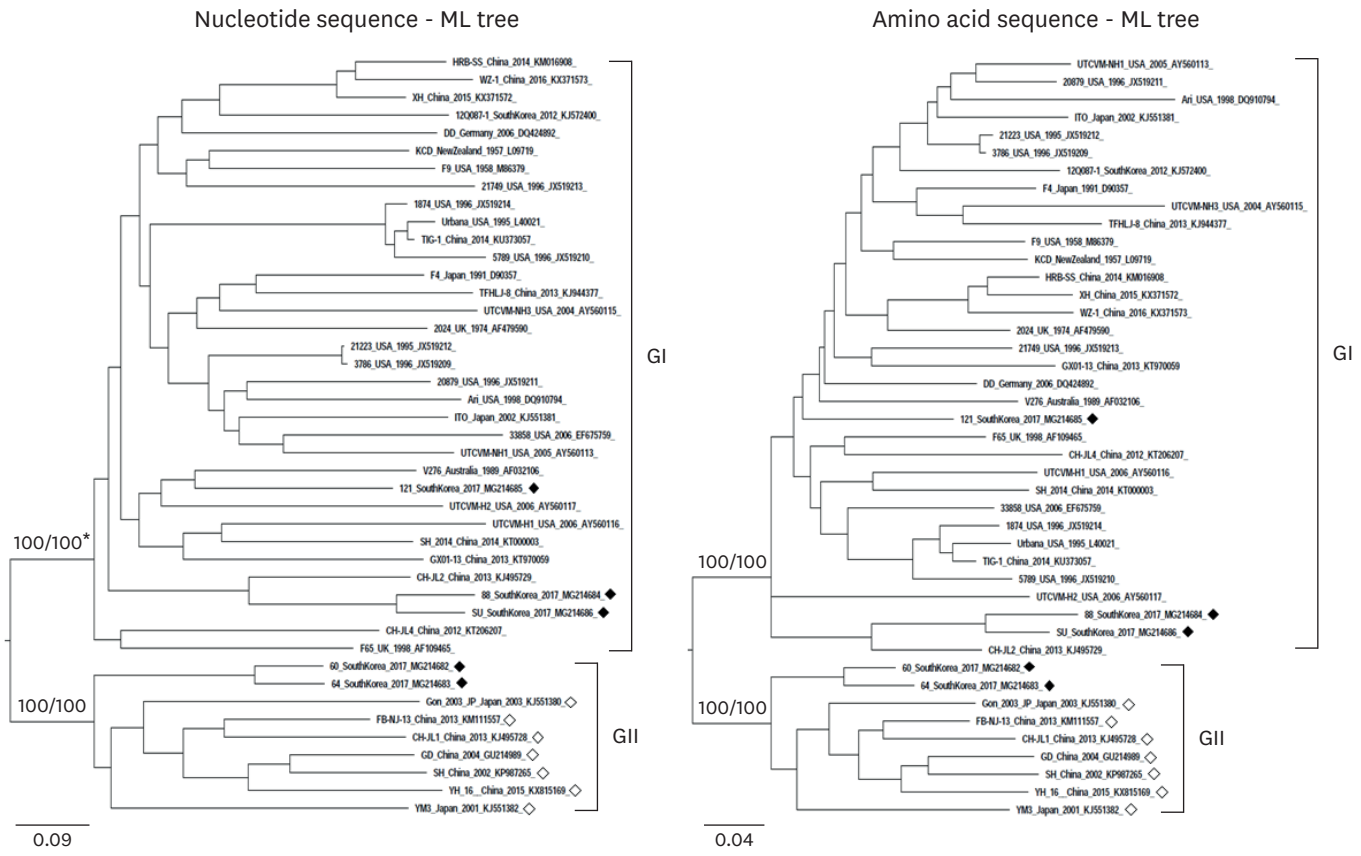

Fig. 2. Phylogenetic tree of FCV isolates based on full-length ORF2 sequences. Closed diamonds mark FCV isolates recovered in this study, and open diamonds mark FCV isolates previously classified into genotype II [3,7].
FCV, feline calicivirus.
*SH-aLRT %/UTBoot %.

The sequence comparison among 59 ORF2 partial sequences including the five new Korean isolates showed distinct variations at 3 aa positions between the two genogroups. Genotype I isolates had Asn/Asp, Ala, and Gly residues, whereas genogroup II isolates possessed Lys, Val, and Ser residues at aa positions 377, 539, and 557, respectively (**Figs. 2** and **3**). In addition, high sequence variability among the FCV isolates including five Korean isolates was observed in the region of the linear B-cell epitope and in the 5′ HVR of region E, compared with the vaccine strains of FCV (F9, F4, and 2024; **Fig. 3**).

DISCUSSION

Owing to the high variability in nt sequences among FCV variants, it has been difficult to generate high-quality PCR products of the ORF2 gene for sequencing [5,6,13]. In this study, the full length ORF2 of five Korean isolates was successfully sequenced using strategically designed novel primer sets binding to conserved regions of ORF1, ORF2, and ORF3 (**Supplementary Fig. 1** and **Supplementary Table 2**). In partitioned triangular graphs generated by likelihood mapping analysis (**Fig. 1**), the three corner regions represent the well-resolved phylogeny (informative) and the central triangle represents a star-like evolutionary pattern (uninformative). The three rectangles represent a partly resolved phylogeny (partly informative). In general, a good dataset should show a high percentage of the sum of the

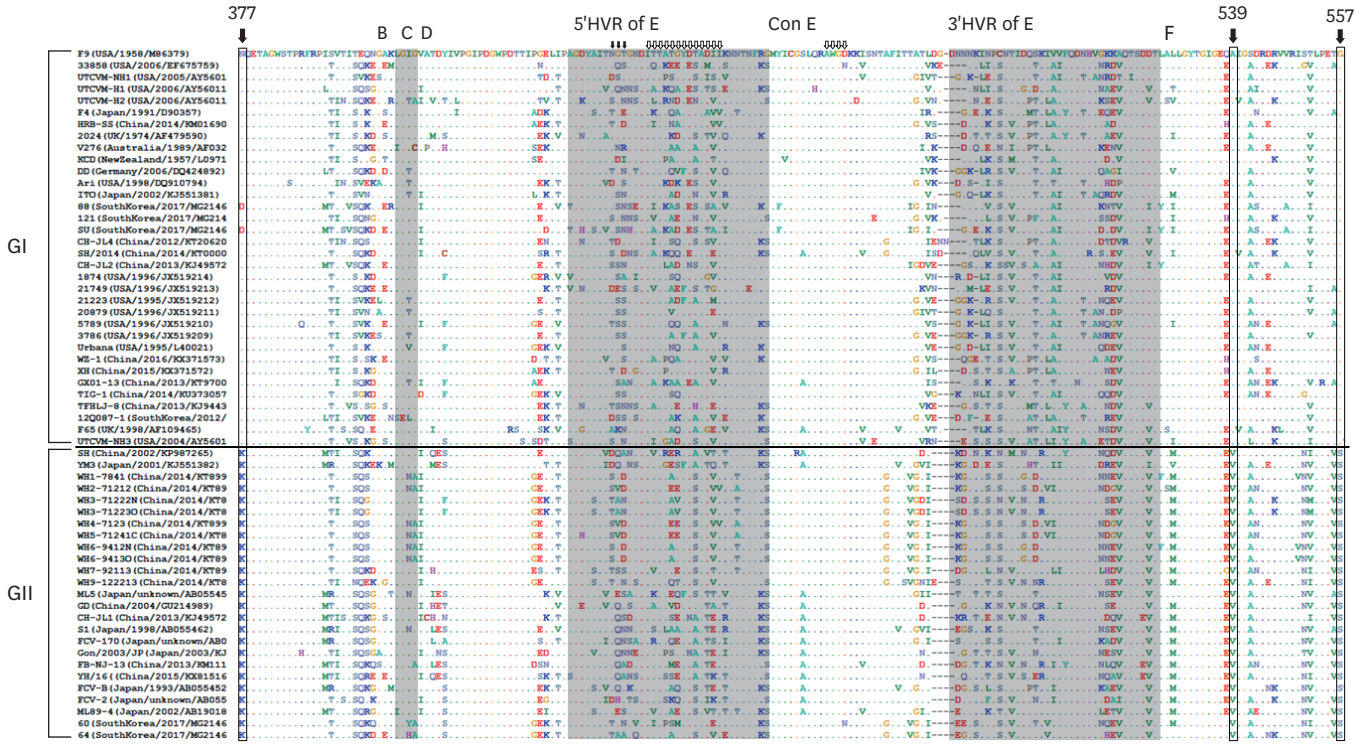

Fig. 3. Amino acid (aa) sequences at position 377–557 in ORF2 of FCV. This sequence contains the partial regions B, C, D, E, and F [1]. Region E consisted of a 5′-end HVR, Con, and 3′-end HVR. Black arrows indicate part of the 5′ HVR of region E with strong immunoreactivity [16]. Open arrows indicate the linear epitopes of F9 [15]. Genotype (G) I and II isolates exhibited Asn (N) or Asp (D)-Ala (A)-Gly (G) and Lys (K)-Val (V)-Ser (S) aa residues at positions 377, 539, and 557, respectively (aa position numbers are based on FCV strain F9).
HVR, high variable region; Con, conserved region; ORF, open reading frame; FCV, feline calicivirus.

three corner regions and a low percentage of the central region [14]. In our analysis, aa sequences were more informative than nt sequences, and the dataset of full-length ORF2 aa sequences was demonstrated to be the best fitting model in topology analysis among the three datasets of nt or aa sequences.

The ML phylogenetic tree based on full-length ORF2 sequences clearly showed two distinct clusters in global FCV isolates including the five Korean isolates. To date, FCV isolates belonging to genotype II have only been reported in Japan and China [3,7]. Therefore, this study demonstrates the presence of both genotype I and II isolates in Korean cats and represents the first report of the isolation of genotype II strains from stray cats. The limited geographical distribution of FCV genotype II isolates in Japan and China has been attributed to the regional proximity of the two countries [3]. Korea is geographically located between Japan and China, which can explain the spread of genotype II strains within the cat population of Korea. Thus, worldwide genotype II FCV strains have been identified only in three adjacent countries in East Asia with no evidence of further spread to other distant continents. These results suggest that genotype II strains are only spreading and circulating locally as endemic strains of East Asia.

Previous studies identified three distinct aa residues (at positions 377, 539, and 557) between genogroup I and II isolates [3,7]. The sequence analysis of the five Korean isolates showed the same results except for isolates 88 and SU (both belonging to genotype I). Isolates 88 and SU had a new variation at aa 377, specifically Asp instead of Asn (**Figs. 2** and **3**). This is the first report of this variant in the genotype I isolates.

https://doi.org/10.4142/jvs.2021.22.e32

ORF2 encodes the major capsid protein VP1 and can be divided into six regions (A–F) based on sequence conservation [1]. Region E has been proposed as an important immunological component owing to the presence of linear B-cell epitopes [15], and the Asn-Gly-Thr sequence (aa 439–441) in the 5′ hypervariable region (HVR) of region E has strong immunogenicity [16]. Therefore, the high sequence variability detected in the region of the linear B-cell epitope and in the 5′ HVR of region E among the FCV isolates (**Fig. 3**) suggests that immunization with a single vaccine strain should be inefficient for protection against FCV infections.

In summary, we further validated the adequacy of classification of FCV strains into genotypes I and II based on phylogenetic analysis of full-length and partial ORF2 sequences of 43 FCV isolates, including five new Korean isolates. Importantly, this study demonstrates the presence of genotype II isolates in Korea, which extends the geographic range from only China and Japan, suggesting that genotype II strains might be endemic strains circulating and evolving only in the limited geographical regions of East Asia. The high sequence variation of the immunogenic components of FCV isolates detected in this study suggests that the current vaccine strains might not be sufficient to protect against infections of variable circulating FCV strains in the cat population, warranting further efforts to develop a novel vaccine for cross-protection.

SUPPLEMENTARY MATERIALS

Supplementary Table 1
Feline calicivirus isolates used in this study

Click here to view

Supplementary Table 2
Primers used for ORF2 sequencing of FCV isolates

Click here to view

Supplementary Fig. 1
Schematic presentation of the full-length ORF2 sequencing strategy. PCR fragments 1 and 2 were purified and directly sequenced with the amplifying primers corresponding to the fragments. PCR fragment 3 was cloned into a plasmid and then sequenced. The full-length ORF2 sequence of each FCV isolate was recovered from the three sequencing datasets.

Click here to view

Supplementary Fig. 2
Frequency distribution of pairwise genetic distances (p-distances) among 43 ORF2 nucleotide sequences. Intra-genotype p-distances and inter-genotype p-distances formed two respective well-bounded areas within a range of 0.008–0.270, although these areas were not completely separated.

Click here to view

Supplementary Fig. 3

Phylogenetic tree of FCV isolates based on the partial ORF2 sequence corresponding to amino acids 288–590. A total of 1000 ultrafast bootstrap (UTBoot) repetitions and the SH-aLRT test were performed to construct each maximum-likelihood tree; the tree was considered to be reliable if SH-aLRT ≥ 80% and UTBoot ≥ 95%. Closed diamonds mark FCV isolates recovered in this study, and open diamonds mark FCV isolates previously classified into genotype II [3,7].

Click here to view

Supplementary Fig. 4

Phylogenetic tree of FCV isolates based on the partial ORF2 sequence corresponding to amino acids 370–583. A total of 1000 ultrafast bootstrap (UTBoot) repetitions and the SH-aLRT test were performed to construct each maximum-likelihood tree. The tree was considered to be reliable if SH-aLRT ≥ 80% and UTBoot ≥ 95%. *SH-aLRT%/UTBoot%. Closed diamonds mark FCV isolates recovered in this study, and open diamonds mark FCV isolates previously classified into genotype II [3,7].

Click here to view

REFERENCES

1. Seal BS, Ridpath JF, Mengeling WL. Analysis of feline calicivirus capsid protein genes: identification of variable antigenic determinant regions of the protein. J Gen Virol. 1993;74(11):2519-2524.
PUBMED | CROSSREF
2. Sosnovtsev SV, Green KY. Identification and genomic mapping of the ORF3 and VPg proteins in feline calicivirus virions. Virology. 2000;277(1):193-203.
PUBMED | CROSSREF
3. Sun Y, Deng M, Peng Z, Hu R, Chen H, Wu B. Genetic and phylogenetic analysis of feline calicivirus isolates in China. Vet J. 2017;220:24-27.
PUBMED | CROSSREF
4. Pereira JJ, Baumworcel N, Fioretti JM, Domingues CF, Moraes LF, Marinho RD, et al. Molecular characterization of feline calicivirus variants from multicat household and public animal shelter in Rio de Janeiro, Brazil. Braz J Microbiol. 2018;49(4):777-784.
PUBMED | CROSSREF
5. Hou J, Sánchez-Vizcaíno F, McGahie D, Lesbros C, Almeras T, Howarth D, et al. European molecular epidemiology and strain diversity of feline calicivirus. Vet Rec. 2016;178(5):114-115.
PUBMED | CROSSREF
6. Afonso MM, Pinchbeck GL, Smith SL, Daly JM, Gaskell RM, Dawson S, et al. A multi-national European cross-sectional study of feline calicivirus epidemiology, diversity and vaccine cross-reactivity. Vaccine. 2017;35(20):2753-2760.
PUBMED | CROSSREF
7. Sato Y, Ohe K, Murakami M, Fukuyama M, Furuhata K, Kishikawa S, et al. Phylogenetic analysis of field isolates of feline calcivirus (FCV) in Japan by sequencing part of its capsid gene. Vet Res Commun. 2002;26(3):205-219.
PUBMED | CROSSREF
8. Kim SJ, Park YH, Park KT. Development of a novel reverse transcription PCR and its application to field sample testing for feline calicivirus prevalence in healthy stray cats in Korea. J Vet Sci. 2020;21(5):e71.
PUBMED | CROSSREF
9. Strimmer K, von Haeseler A. Likelihood-mapping: a simple method to visualize phylogenetic content of a sequence alignment. Proc Natl Acad Sci U S A. 1997;94(13):6815-6819.
PUBMED | CROSSREF

10. Minh BQ, Nguyen MA, von Haeseler A. Ultrafast approximation for phylogenetic bootstrap. Mol Biol Evol. 2013;30(5):1188-1195.
PUBMED | CROSSREF

11. Kumar S, Tamura K, Jakobsen IB, Nei M. MEGA2: molecular evolutionary genetics analysis software. Bioinformatics. 2001;17(12):1244-1245.
PUBMED | CROSSREF

12. Hsiao KL, Wang LY, Lin CL, Liu HF. New phylogenetic groups of torque teno virus identified in eastern Taiwan indigenes. PLoS One. 2016;11(2):e0149901.
PUBMED | CROSSREF

13. Coyne KP, Edwards D, Radford AD, Cripps P, Jones D, Wood JL, et al. Longitudinal molecular epidemiological analysis of feline calicivirus infection in an animal shelter: a model for investigating calicivirus transmission within high-density, high-turnover populations. J Clin Microbiol. 2007;45(10):3239-3244.
PUBMED | CROSSREF

14. Zhaxybayeva O, Gogarten JP. Bootstrap, Bayesian probability and maximum likelihood mapping: exploring new tools for comparative genome analyses. BMC Genomics. 2002;3(1):4.
PUBMED | CROSSREF

15. Radford AD, Willoughby K, Dawson S, McCracken C, Gaskell RM. The capsid gene of feline calicivirus contains linear B-cell epitopes in both variable and conserved regions. J Virol. 1999;73(10):8496-8502.
PUBMED | CROSSREF

16. Seal BS. Analysis of capsid protein gene variation among divergent isolates of feline calicivirus. Virus Res. 1994;33(1):39-53.
PUBMED | CROSSREF